T2 RELAXATION PARALLELS PROGRESSIVE CLINICAL SYMPTOMS OF DEMYELINATION IN A NOVEL TRANSGENIC MOUSE MODEL

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INTRODUCTION

Loss of myelinating cells is a common hallmark of demyelinating diseases such as Multiple Sclerosis (MS), and its impact on axonal damage is still poorly understood. A novel mouse model of demyelination has been generated semi-spontaneously by knock-in expression of a diphtheria toxin fragment A (DT-A) in the oligodendrocyte specific Cre-expressing line [4], allowing spatially and temporally controlled triggering of oligodendrocyte death. This model will further enhance insights into disease mechanisms triggered by demyelination. Axonal damage seems to be the major disability correlate in these diseases - contrary to primary affection of myelinating glial cells.

Sensitively specific, non-invasive imaging marker for monitoring de- and remyelination in vivo would be of paramount importance. To date, there is no established MRI marker for assessing demyelination, although quantitative magnetization transfer ratio (qMTR) MRI [1] and Relaxivity-weighted MRI [2] has shown high sensitivity in detecting myelin pathology. Similarly the use of iron-oxide nanoparticles (USPIOs) to visualize macrophage infiltration has been proposed as biomarker for MS and animal models thereof [3], primarily reflecting inflammatory events. To evaluate the sensitivity of various MR readouts for monitoring demyelination pathology we semi-naturally assessed T2, MTR and infiltration of macrophages using USPIOs in control and diseased mice over the time-course of five weeks.

METHODS

In vivo experiments were carried out in strict adherence with the Swiss law for animal protection.

Mouse model: Generated by crossing a tamoxifen (TAM) inducible, oligodendrocyte specific Cre expressing mouse line [4] with a mouse line expressing diphtheria toxin fragment A (DT-A) [5] allowing spatially and temporally controlled induction. Induction was achieved in mice expressing tamoxifen (TAM) for 5 consecutive days (start at d=1). The study design comprised 2 experimental groups: group1: recording of MR T1 and T2 maps; group2: recording of T2 maps prior- and 24h post USPIO (Sinergen®, Guebert, France) injection (i.v. 340μM Fe/kg). Each experimental group consisted of 2 control (no TAM injection: -TAM) and 4 mice which received ip. injection of TAM (i.p. dosing/kg: +TAM) Longitudinal MRI measurements of group 1 were performed at days 3, 10, 17, 24, 31, 38 and 42. Animals of group 2 received USPIOs immediately after the first TAM acquisition (pre-USPIO) and were followed up 24 h later (post-USPIO). All MRI experiments were conducted on a Bruker Bielspin MRI (Bruker BioSpin MRI, Eningen, Germany) small animal system operating at 200 MHz. MRI measurements were performed using a birdcage transmit/receive resonator. Mice were anesthetized using 1.8% isoflurane applied in a face mask in an oxygen (20%:80%) mixture and placed on water-heated plate. MTR Transfer imaging sequence (MTR) was performed for a 3D-GE sequence (FOV: 10x10x12mm; acquisition matrix: 512x512x32; isotropic voxel size of 125μm; TE/TR: 2.030/1.030ms; flip angle 30°). A gausian pulse with B1 amplitude of 80μT and an offset frequency of 5 kHz was used at MR saturation pulse. The reference sequence was obtained with the same parameters but without application of the saturation pulse. Accordingly the MTR was calculated as: MTR [%] = (M(0) - M(T)) / M(0) × 100, where M(0) is the magnitude of signal without MR saturation and M(T) is the magnitude of signal during MR saturation. MTR values were obtained using a multi-echo-echo sequence with the following parameters: FOV: 12x12x12; in plane resolution of (144μm); TE/TR: 10.000000ms; echo spacing 10.0 ms; N°: echoes: 14; NA: 6; and SLTH: 0.8mm). High resolution anatomical images were acquired using a standard SE sequence with FOV: 20x24; acquisition matrix: 512x512 in plane resolution of 75x75μm; TE/TEeff/TE: 12.000000ms; RARE factor: 8; NA: 7; SLTH: 0.8mm; N°: 9 echoes: 9. Image analyses: T1 and T2 MRI maps were calculated using homemade software (M.Rausch, Novartis, Switzerland). All calculated parametric T2 maps from 9 animals were averaged and evolution of T2 values plotted over time for different ROIs (frontal cortex: tcor; olfactory bulb: obtb; cerebral white matter: cwbm; brainstem: bstem, Fig.2a). The effect of TAM injection was calculated as the difference (ΔT2) between T2(24h post) - T2(pre) injection of USPIOs for all timepoints (d4, 11, 18, 25, 32 and 39). Computed MTR maps were resliced and coregistered to the high-resolution images. ROIs were defined in bstem, cwbm and bstem. All MR data are given as means±SEM. Time-courses were statistically analyzed using Repeated measures ANOVA (treatment: +TAM, and Fisher’s PLSD post-hoc test (p<0.05).

RESULTS

Analysis of T2 maps shows pronounced increase in T2 in +TAM mice. +TAM animals in brain regions affected by demyelination as revealed by histology i.e. cerebellar white matter and brainstem (Fig.1). Significant differences between the two groups were observed for cwbm (treatment x time interaction: F(6, 24) = 12.8, p<0.001) and bstem (treatment x time interaction: F(6, 24) = 3.79, p<0.0085). The temporal evolution of T2 changes was similar in +TAM mice and in +TAM mice with absent DT-A as indicated by demyelination score Fig.2. No change in T2 was observed in control brain regions not affected by the disease. The earliest time-point where a significant increase in T2 was observed in +TAM mice in control brain regions is visible on T2 maps for the regions analysed (Fig.3a) indicating the integrity of the blood-brain barrier for USPIOs and absence of pronounced active macrophage infiltration over the time-course of the experiments. Similarly only small decreases in MTR could be detected in a progressed pathological stage (Fig.4a). Fig.4b shows colour coded MTR maps at the level of bstem for baseline (d3) and end-stage (d42) for both groups.

DISCUSSION

T2 hyperintensities become apparent at the time-point of first clinical symptoms reflecting the massive, even lesion-like damage which occurs alongside white matter tracts of the CNS. Increased T2 values are indicative of edema formation. In view of these early non-invasive readouts for this documented feature the assessment of T2 may serve as a valuable biomarker of the disease process. On the other hand a decrease in MTR could only be detected at a late stage in pathology probably reflecting the late loss of myelin macromolecules rather than their early structural disruption. No active or passive infiltration of macrophages could be measured reflecting the intact blood-brain-barrier (Fig.3b) and the weak recruitment of blood-borne immune cells described for this intriguingly modelled of demyelination.

REFERENCES